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CARYCINEL RED AND COCCINEL RED, OIL SOLUBLE ANTHRAQUINONE DYES, AS FAT STAINS IN ANIMAL HISTOLOGY

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The purpose of this communication is to report two new red oil soluble dyes which appear to be superior as fat stains to those now in use.

Following our work with oil blue NA,[1] we raised the question as to whether there might not be also some red dyes of similar chemical constitution which would share the property shown by that dye of staining promptly from dilute alcoholic solutions. The National Aniline Division kindly furnished us with two oil soluble red anthraquinone dyes, designated simply as Nos. 1 and 2.

Since these names were not deemed sufficiently distinctive, and since the name oil red 2R is a synonym for naphthylamin Bordeaux (C.I. No. 82), and oil red 3BX and oil red IV are synonyms for Sudan IV (C.I. No. 258), it seemed desirable to propose new names for these dyes.

For No. 1, the name "carycinel red" is proposed, from *καρύκινος*, blood red, and *ἔλαιον*, oil, and for No. 2, the name "coccinel red", from *κόκκινος*, scarlet and *ἔλαιον*, oil. In color carycinel red is a deep, pure red, and coccinel red, a deep orange scarlet, similar to oil red O. Both contrast well with alum hematoxylin nuclear stains.

For carycinel red, the following spectroscopic data are presented. λ = 520 mμ, the band of 90% of maximum optical density was 40 mμ wide, with median at 523. Optical density (D) was 0.316 in a 10 mm. cell in a 1:100,000 (10 mg. per liter) solution in ethyl alcohol. Chemically it is 1-amylaminoanthraquinone.

Corresponding data for coccinel red were λ = 512 mμ, 90% D band width 41 mμ, median 510. D = .287 at 10 mg. per liter in

[1]Lillie, R. D. Oil blue N or NA as a fat stain in animal histology. Stain Techn. 20, 7–9. 1945.

ethyl alcohol in a 10 mm. cell. Chemically, it is 1,5-diamylaminoanthraquinone.

Solutions of each of these were made in 99% isopropanol, using .5 gm. dye to 100 ml. solvent. The residues were filtered out onto previously weighed filter papers, dried and weighed. That from carycinel red weighed 735 mg., that from coccinel red, 795 mg., indicating solubilities of 4.2 to 4.3%.

Dilutions of each of these stock solutions were made with distilled water so as to yield solutions in 90, 80, 70, 60, 50, 40, 30, 20 and 10% isopropanol. Frozen sections of normal cat kidney and of human adrenal were stained 10 minutes in each of these dilutions. As expected, considerable loss of fat was noted in the first three, in 90 to 70% isopropanol. Satisfactory staining was attained in the 60 to 30% isopropanol solutions, with both dyes, and with carycinel red, fairly good staining from the 20% isopropanol solution.

These dilutions were all used within 30 minutes of dilution from the stock solution in 99% isopropanol, hence were in the supersaturated state.

Next the oil blue N technic was tried, in which a stock solution saturated in 60% isopropanol was diluted for use to the 40% isopropanol level. With both dyes paler red stains were attained than with the 30% isopropanol dilutions from the 99% isopropanol stock solution. Immersion of sections in these 40% isopropanol solutions for 6 hours occasioned no evident loss of fat.

A series of tests was then made as to the stability of the fresh dilutions of the 99% isopropanol stock solutions of the two dyes to 20, 30 and 40% isopropanol levels. Dilutions were allowed to stand 7 or 8 minutes, and then filtered. Frozen sections were stained 10, 20 and 30 minutes at intervals of 10 minutes and 1, 2, 3, 4, 5 and 6 hours after dilution.

With 40% isopropanol, carycinel red gave unimpaired staining for 6 hours in 10 minutes, while with coccinel red, staining became weaker after 2 hours, and better results were obtained by prolonging staining time to 20 minutes, or after 5 or 6 hours to 30 minutes.

At 30% isopropanol level, both gave quite good staining in the first hour, but after 5 hours, carycinel red was ineffective, while coccinel red required 20 minutes for satisfactory staining at 2 to 6 hours after dilution.

With 20% isopropanol, carycinel red was ineffective at 10 minutes after dilution, while coccinel red gave fair to good results in 20 to 30 minutes staining throughout the 6-hour period.

Some precipitation is observed with carycinel red, especially

from 40% isopropanol staining solutions, while with coccinel red, practically no precipitation was observed from solutions in less than 60% isopropanol.

Summary

Carycinel red, 1-amylaminoanthraquinone, and coccinel red, 1,5-diamyldiaminoanthraquinone, give excellent deep red and deep orange-red fat stains in 10 to 20 minutes from fresh dilutions in 30 or 40% isopropanol in water, prepared from stock saturated solutions (about 4.2%) in absolute isopropanol. The diluted staining solutions can be used for several hours only. With 40% isopropanol loss of fats is inappreciable in 6 hours, while only 10 to 20 minutes is required for staining.

The author is indebted to Medical Technician R. R. Faulkner for technical assistance.

STAINING A SPRAY FILM ON CONIFER NEEDLES

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As a preliminary step in an investigation designed to reveal the coverage of a spray film on conifer needles after various periods of weathering, it was necessary to find a microtechnic which would

FIG. 1. Needle of *Pseudotsuga taxifolia* with spray film. Cross-section, Magnification 1000×.

leave the spray coat unaltered, and yet differentiate it from the underlying tissues. The usual operations of killing and embedding were, of course, not applicable.

The particular spray material used in this study was developed to reduce transpiration from foliage. It was an oil-in-water emulsion, prepared with 7.5% plant oils of quick drying properties (mixture of linseed, soybean and castor oils) and with small amounts of ammonium salts of fatty acids forming the emulsifying system.

Fig. 2. Needle of *Pinus densiflora* with spray film. Oil emulsion penetrated into the stomatal pit. Cross-section. Magnification 1000×.

The procedure which has proved successful with this spray emulsion is described below. It may be applied also to other problems concerned with similar protective agents.[1]

[1]The spray material underwent minor changes in its composition since this study has been carried out. However, the staining method described is still applicable. The emulsion will be commercially available in the spring of 1945 for use in orchards.

Method

1. Hold needles on which the spray has completely dried between elder pith; make free-hand cross-sections.
2. Float sections in distilled water.
3. Transfer sections singly into Eau de Javelle (potassium hypochlorite solution) and leave therein until properly bleached (about 20 minutes).
4. Wash in distilled water.
5. Immerse for a few seconds in a slightly acidified 0.2% solution of Nile blue sulphate[2] in 25% alcohol.
6. Wash 1 minute in 70% alcohol.
7. Counterstain 10 to 15 minutes in a saturated solution of Sudan IV[2] in 70% alcohol.
8. Remove excess stain by washing a few seconds in 50% alcohol.
9. Wash in distilled water.
10. Mount in 50% glycerin.
11. Replace the diluted glycerin by pure glycerin before sealing the preparation.

Care is required in transferring the leaf sections from one fluid to another as there is danger of the spray film being lost or altered through too rough manipulation.

Results

The spray film—dark red; the cuticle—brownish red; the epidermis—green; the mesophyll—blue.

The outlined procedure makes it possible to test the coating and penetrating character of oil emulsion films on leaf surfaces. However, a great number of sections must be made in order to obtain a complete picture of the distribution of the film on the whole leaf area.

Photomicrographs of preparations just described are shown in Figs. 1 and 2.

[2]The stains used by the writer were obtained from Coleman and Bell.

A BUFFER MODIFICATION OF THE WARTHIN-STARRY SILVER METHOD FOR SPIROCHAETES IN SINGLE PARAFFIN SECTIONS

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The Warthin-Starry[1] procedure, according to Kerr[2], appears to be one of the more reliable methods for demonstration of spirochaetes in single paraffin sections. However, the adjustment of the pH of the distilled water used to pH 4.4 with citric acid necessitates the use of indicator each time, and, further, the citric acid stock solution has to be made fresh at frequent intervals because of its susceptibility to mold growth.

For these reasons it seemed desirable to find a suitable buffer for maintenance of the desired pH level. Walpole's sodium-acetate–acetic-acid buffer and a sodium-citrate–citric-acid buffer were found suitable in that they do not precipitate silver salts. The latter is made up in 25% alcohol, which effectively prevents growth of molds.

Walpole's[3] buffer solutions are fifth molar solutions of sodium acetate (16.4 g. per liter) and acetic acid (12 g. or 11.8 ml. per liter) in distilled water; 17.6 ml. of the acetic and 2.4 ml. of the acetate give pH 3.8; 18.5 and 1.5 respectively give pH 3.6. These are after Clark's[4] tables, and vary slightly from Walpole's original data.

The sodium-citrate–citric-acid series is made with tenth molar solutions of sodium citrate (29.4 g. per liter) and of citric acid (21 g. per liter), both in 25% ethyl alcohol[5]. A mixture of 1 ml. sodium citrate and 19 ml. citric acid gives pH 2.95, the citric acid alone

[1]WARTHIN, A. S., and STARRY, A. C. 1920. A more rapid and improved method of demonstrating spirochaetes in tissues. Amer. J. Syph., **4**, 97.

FARRIER, R., and WARTHIN, A. S. 1930. A study of the effect of pH upon the third improved Warthin-Starry method for demonstrating *Spirochaeta pallida* in single sections. Amer. J. Syph., **14**, 394.

[2]KERR, D. A. 1938. Improved Warthin-Starry method of staining spirochaetes in tissue sections. Amer. J. Clin. Path., **8**, 63.

[3]WALPOLE, G. S. 1914. Hydrogen potentials of mixtures of acetic acid and sodium acetate. J. Chem. Soc., **105**, 2501.

[4]CLARK, W. M. 1922. The determination of hydrogen ions. (Table 29, p. 115), *2nd Ed.* Williams & Wilkins Co., Baltimore, Md.

[5]The sodium-citrate–citric-acid series was devised in this laboratory by Dr. R. D. Lillie and measured electrometrically by Dr. B. L. Horecker of the Industrial Hygiene Research Laboratory of this Institute.

somewhat lower (2.87). Both of these buffers at both levels have given good results, but the acetate series seems somewhat better.

The technic using the acetic-acid–sodium-acetate buffer follows:

1. Paraffin sections, on 1×3 slides, are brought through xylene and graded alcohols to water buffered to pH 3.6 or 3.8 by addition of 20 ml. of mixed acetate buffer to 480 ml. distilled water.

2. Impregnate sections 45 minutes at 55–60°C. (in paraffin oven) in 1% $AgNO_3$ in water of the same buffer level.

3. Place slides face up on glass rods and pour on developer previously heated to 55–60°C. When sections become golden brown to grayish yellow and developer begins to turn brownish black (3–5 minutes), pour off and rinse with warm (55–60°C.) tap water, then with distilled water.

4. Dehydrate, clear and mount in xylene clarite.

All glassware used should be chemically clean. Coplin jars are used for the oven impregnation of slides.

To make the developer, heat 15 ml. 5% gelatin in the above dilute buffer solution to 60°C. Just before using, add 3 ml. 2% $AgNO_3$ and 1 ml. 3% hydroquinone, both also dissolved in the same buffer solution. Avoid direct sunlight and cold drafts when using.

The stock gelatin solution can be made by dissolving 10 g. gelatin in 200 ml. water buffered to pH 3.6 or 3.8 as above in the paraffin oven for 1 hour. Addition of 2 ml. 1:10,000 merthiolate is necessary if the solution is to be used over a period of several days. The stock 1% and 2% $AgNO_3$ solutions can be kept in brown glass bottles in the dark. The 3% hydroquinone should be made fresh daily (300 mg. in 10 ml. buffered water should be adequate). All solutions should be warmed before mixing.

Under-development gives pale background and very slender or pale spirochaetes, over-development gives dense background, heavily impregnated spirochaetes with obscuration of detail and, often, precipitates. Lower pH levels tend to decrease staining of both spirochaetes and background, higher to increase. The chosen levels of 3.6 to 3.8 give pale yellowish brown tissue staining and moderately heavy black spirochaetes, the lower less tissue staining, the higher denser organisms.

The method has been used successfully on syphilitic fetal liver, on yaws lesions, on liver and kidney of animals infected with various leptospiroses and other tissues, including Vincent's stomatitis.

If it is desired to counterstain sections with hematoxylin and eosin or other methods, it is necessary to first tone sections in 1:500 acid gold chloride ($HAuCl_4 \cdot xH_2O$) for 3 minutes. This follows step 3 of the technic.

PREPARATION OF BLOOD SMEARS WITH THE FORMALIN VAPOR METHOD

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It is known that dry smears do not preserve all blood elements and the varieties destroyed by this method increase in descending to lower animals. In Amphibia—to which the present communication applies in particular—spindle cells (thrombocytes) and later basophils invariably disintegrate, other elements suffering more or less damage due to contact with air and the strain on the relatively liquid cells.

To overcome this difficulty, wet smears, release of blood into the fixative, or fumigation of the smears, have been recommended. Of these, the first is not greatly superior to the dry method. Letting the blood flow directly into the fixative and then, after various procedures, spreading it on a slide, preserves all the elements well; but besides being laborious, this technic has the serious disadvantage that the leucocytes, remaining contracted, are usually inaccessible for a study of their granulations and finer structures. By far the best results obtained in our experience are with fumigation methods.

Malassez (1882, quoted from Weidenreich) was first to treat smears with fumes of osmic-acid. Weidenreich[1] improved the method by exposing the slides to an additional fumigation, prior to spreading the blood, which instantly kills and partly fixes the cells upon contact with the glass. He also recommended the use of formalin.

The following is a further improvement of the formol method and yields results equal or even superior to the osmic technic. It is here offered in two modifications of fixation, each producing somewhat different staining effects.

Fixation:

(a) Place slides on a rack mounted on a Petri dish containing a few milliliters of formalin and cover all with a larger dish or bell. After 5–10 min. (or longer) remove slides, quickly prepare a smear on the side exposed to the fumes and replace, with blood-film facing formalin; leave in fumes for 5–10 min., remove and flame 5 times; as soon as slides have cooled, stain.

[1]Weidenreich, F. 1906. Studien über das Blut und die blutbildenden und zerstörenden Organe. IV. Arch. mikr. Anat., **69**, 389–438.

(b) Proceed as under (a), omit flaming and let preparations dry for 6–24 hr., then stain. The latter is only a slight modification of Weidenreich's technic and like it does not preserve the basophils as well as the previous modification. Extending the second fumigation to 15–20 min. gives somewhat better results.

Staining:

For Amphibia use the following modification of Maximow's azure-eosin: H_2O, 100 ml.; 0.1% aqueous azure II, (Grübler) 5 ml.; 0.1% aqueous eosin Y, (Grübler) 7.5 ml.

(a) Stain ..... 6 hr.
(b) Differentiate in 95% alcohol ..... 1–2 min.
(c) Absolute alcohol ..... ½–1 min.
(d) Xylene (3 changes)
(e) Mount

For Wright's stain the procedure is as follows:

(a) Stain in 1 ml. of stock: 100 ml. H_2O .... 4–6 hr.
(b) Rinse in H_2O ..... 30 sec.
(c) Dehydrate in acetone ..... 30–60 sec.
(d) Xylene, etc.

Results:

Following fixation (a), the plasma of erythrocytes stains a light green color, the leucocytes in the usual manner. The contrasting coloration of the erythrocytes facilitates estimation of color nuances in granulations and is easy on the eyes. Fixation (b) renders the erythrocytes the usual pinkish color.

Numerous other stains were successfully applied following formalin fumigation: thionin, Unna's Blue Polychrome (a polychromed mixture of methylene blue and toluidine blue O), hematoxylin, etc.

Modification (a) is applicable to other vertebrates and man and for some purposes—such as the study of lymphocytes—seems to be superior to the dry-smear technic.

STAINING OF MUCUS WITH BUFFERED SOLUTIONS OF TOLUIDINE BLUE O, THIONIN AND NEW METHYLENE BLUE N

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ABSTRACT.—**The addition of buffer mixtures to toluidine blue O, thionin and new methylene blue N improves their use in the staining of mucus after formaldehyde fixation. Overstaining is minimized. The buffer mixtures used consisted of variable proportions of *M*/10 citric acid and *M*/5 anhydrous Na_2HPO_4 in 25% methanol. Connective tissue mucus stained satisfactorily with these dyes at a buffer pH range of 3.4 and 3.95 and epithelial mucus at a range of 2.2 to 3.95. The corresponding ratios of *M*/10 citric acid: *M*/5 Na_2HPO_4 are 16:4 to 14:6 and 20:0 to 14:6 respectively.**

The use of new methylene blue N for staining mucus has not, so far as known, been described previously in the literature. Mucus is stained purplish red and in striking contrast to a violet blue background.

The staining solution most commonly used at this Laboratory for demonstrating mucus in microscopic preparations is 1:1000 aqueous toluidine blue O.[1] This makes a very satisfactory stain when applied to paraffin sections for 30 to 45 seconds, but if the sections are stained for more than a minute, there is frequently considerable overstaining with poor cellular detail and differentiation and obscuring of mucus. An attempt was made to correct this fault by the use of buffer mixtures.

To a 1:500 aqueous solution of the dye was added an equal volume of various buffer solutions consisting of graded mixtures of *M*/10 citric acid and *M*/5 anhydrous Na_2HPO_4 in 25% methanol. The pH of such mixtures was determined electrometrically and tabulated in another paper.[2] Serial sections of a formalin-fixed mammary gland showing extensive mucoid degeneration of the lobular stroma were used to study connective tissue mucus. A formalin-fixed

[1]Lillie, R. D. 1928. Eine Schnellmethode zur Toluidinblau-Schleimfärbung. Zts. wiss. Mikr., 45, 381.

[2]Highman, B., and Lillie, R. D. 1944. Romanowsky staining with buffered solutions. IV. Influence of fixatives on buffered Romanowsky. J. Techn. Methods, 24, 31–4.

appendix was used similarly to study epithelial mucus. The buffered staining solutions were not considered satisfactory unless they gave good results when applied to the sections for periods varying from 30 seconds to one hour. After staining, the sections were washed in water, dehydrated, cleared and mounted by successive use of acetone, acetone and xylene mixture, xylene, and clarite. Two lots each of toluidine blue O and thionin and one lot of new methylene blue N were tested.[3]

With toluidine blue O, the color of the mucus, especially in the epithelium, often varied considerably in the same section. In general, it ranged from reddish purple with the more acid to violet blue with the less acid buffer mixtures. With thionin, the mucus was less blue but not as well contrasted. The color imparted to various tissue elements in sections stained with new methylene blue N was intermediate between that of thionin and toluidine blue O. Mucus was commonly stained purplish red and in striking contrast to a violet blue background. The reddish hue was usually more pronounced when more acid buffers were used.

The use of new methylene blue N for staining mucus has not, so far as known, been described previously in the literature. Cowdry[4] used it as a vital dye for staining Nissl bodies of spinal ganglion cells. According to Conn[5], it appears probable that a sample of new methylene blue N mislabelled methylene blue was used by Louise Smith[6] in staining cartilage by the Van Wijhe technic. Lillie[7] described tinctorially its staining of nuclei, bacteria, and mast cell granules when used in combination with various fluorane dyes. He did not record its staining of mucus when so used or its tinctorial qualities when used alone, but subsequently suggested verbally to the writer that its possibilities as a stain for mucus be investigated.

All five dye samples were found to require similar buffer pH values. Connective tissue mucus was stained satisfactorily by these dyes when the pH of the buffer mixture varied from 3.4 to 3.95 (ratio

[3]The dye samples tested were thionin C.I. No. 920, NT-1 and NT-8, toluidine blue O, C.I. No. 925, NU-2 and Biosol Products Co. No. 162, and new methylene blue N, C.I. No. 927, NAC No. 3217 (National Aniline Division). The lot designations NT-1, NT-8 and NU-2 are those of the Biological Stain Commission.

[4]Cowdry, E. V. 1914. The comparative distribution of mitochondria in spinal ganglion cells of vertebrates. Amer. J. Anat., **17**, 1.

[5]Conn, H. J. 1925. Biological Stains, *1st Ed.* Biotech Publications, Geneva, New York.

[6]Smith, Louise. 1920. The hyobranchial apparatus of *Spelerpes bislineatus*. J. Morph., **33**, 527.

[7]Lillie, R. D. 1944. Various thiazine and fluorane dyes in pair combinations as neutral stains for tissue. J. Techn. Methods, **24**, 43–5.

of *M*/10 citric acid: *M*/5 Na_2HPO_4 varied from 16:4 to 14:6). At higher pH levels there was eventual diffuse overstaining. At lower levels connective tissue mucus exhibited little or no metachromasia. Epithelial mucus stained satisfactorily when the buffer pH range was 2.2 to 3.95 (ratio of *M*/10 citric acid: *M*/5 Na_2HPO_4 20:0 to 14:6). With more alkaline mixtures it did not stain appreciably, or was obscured by protoplasmic overstaining. However, even at considerably lower pH levels, using acetic and other acids, the epithelial mucus was excellently stained and contrasted although the cells, particularly the cytoplasm, were understained.

Both epithelial and connective tissue mucus were well stained when the *M*/10 citric acid: *M*/5 Na_2HPO_4 ratio was 15:5, or pH 3.7. During the past year we have stained a great variety of surgical and autopsy sections with toluidine blue O buffered in this manner and found it satisfactory for demonstrating mucus. More recently we have been using a similarly buffered solution of new methylene blue N with satisfactory results.

Although we commonly employed stain dilutions of 1:1000, almost equivalent results were secured with dilutions of 1:100 to 1:3000. Likewise, the percentage volume of the buffer mixture could be reduced substantially without altering the staining materially. The use of ethyl alcohol up to 20% by volume in the staining solution as a preservative did not appreciably affect the staining, but its use as a dehydrating agent in place of acetone was detrimental, particularly in reference to epithelial mucus.

Since various stain lots may vary slightly in optimum pH level for staining and since buffer values may also vary slightly with different batches of stock buffers, each fresh lot of stain or of buffer should be checked by staining a number of sections from the same block at several pH levels in the expected range, and thereby the preferred level may be selected.

A SCHEDULE INCLUDING COLD TREATMENT TO FACILITATE SOMATIC CHROMOSOME COUNTS IN CERTAIN FORAGE GRASSES[1]

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ABSTRACT.—**Pretreatment at low temperature of root tips of grass grown from tillers in small beakers was found to contract somatic chromosomes and thus facilitate determination of chromosome number. A modification of Randolph's infiltration and card technics has been adapted to working with root tips from large numbers of plants.**

In a study of the origin of aneuploidy in orchard grass (*Dactylis glomerata*), previously published methods of preparing root tips for chromosome counts proved unsatisfactory. Because of the relative length and tendency for overlapping of the somatic chromosomes, exact determinations of chromosome number were obtained only with great difficulty. Pretreatment of the root tips in Carnoy's solution and in colchicine solution were ineffective. Likewise, modifications of the fixing solution, although resulting in improved preparations in some cases, did not yield entirely satisfactory results. It was discovered, however, that exposure of the plants to low temperature just previous to fixing caused contraction of the chromosomes which made for greater precision in the counts from metaphase plates.

To secure root tips for determination of chromosome number the plants are grown in pots in the greenhouse. When several tillers have developed on each plant, one to three tillers are broken off, the old roots trimmed away, and the tillers placed in tap water in a 150 ml. beaker for the production of new root tips. Tillers from 8 to 10 plants may be placed in a single beaker. The remainder of each plant is repotted and retained for further study if desired. In the greenhouse with a daily change of water to a depth of 12 to 18 mm., satisfactory growth ensues without addition of nutrients. When a desired number of roots has grown to 10 or 12 mm. in length, the beakers are removed to a low temperature chamber. This method of securing root tips from tillers in beakers has two advan-

[1]Contribution No. 73, of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, State College, Pennsylvania, in cooperation with the northeastern states.

[2]Scientific Aid and Geneticist, respectively.

tages. The root tips obtained are younger, straighter, and freer of soil than those produced on plants growing in pots. Also, there is one beaker instead of 8 to 10 pots to move to the cold chamber.

With orchard grass, exposure to 2° C. for 24 hours effects the desired change in the chromosomes and at the same time leaves a sufficient number of nuclei in metaphase stages. Longer and shorter periods were unsatisfactory. It is assumed that the lower temperature reduces the rate of division and results in contraction of the chromosomes. The cold treatment has also been used successfully with meadow and tall fescue.

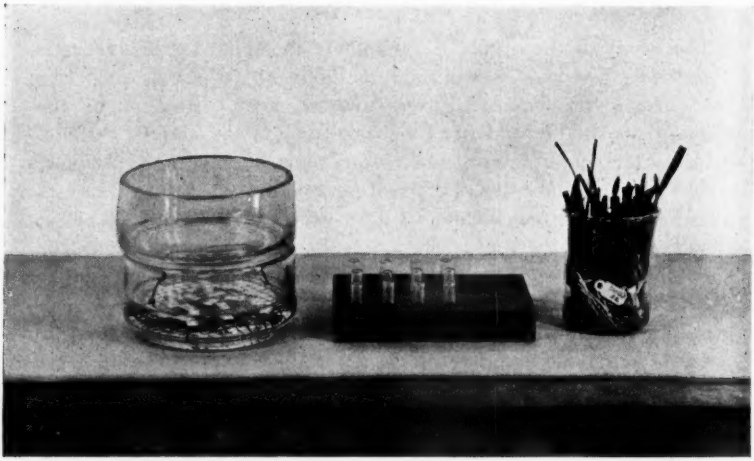

FIG. 1. Tillers with young roots growing in beaker, miniature vials for fixing solution supported in wooden block, and preparation dish with wire tray holding root tips glued to labelled cards.

To facilitate the handling of root tips from a large number of plants, Randolph's[3] card technic is used, modified by omission of the first card. For individual fixing of roots from different plants, 25 × 8 mm. vials supported in indentations in small wooden blocks are very convenient (Fig. 1). The blocks and position on each block are numbered to aid in maintaining the identity of the tips from individual plants. Trials with modifications of Navaschin solution[4] eventually resulted in the use of the following formula in which acetic

[3]Randolph, L. F. Card mounts for handling root tips in the paraffin method. Stain Techn., **15**, 45–8. 1940.

[4]Myers, W. M. Colchicine induced tetraploidy in perennial ryegrass, *Lolium perenne* L. J. Hered., **30**, 499–504. 1939.

acid has been replaced by propionic acid. At time of using, solution A (7 ml. of 10% chromic acid, 15 ml. propionic acid, and 78 ml. distilled water) is mixed in equal proportions with solution B (30 ml. formalin, 60 ml. distilled water, and 10 ml. of 95% ethyl alcohol).

When the root tips have been fixed for 20 to 24 hours, they are brushed clean with a fine brush in a Petri dish of water, cut to the desired length and arranged in orderly fashion on blotting paper wet with 50% alcohol. They are then ready to be glued to the labelled 7 × 12 mm. cards in suitable order for subsequent identification in the manner described by Randolph[3]. This procedure is facilitated if the card at first has a mere trace of glue and then, when the root tips have been placed, a generous drop of glue is spread over them before transferring the card and tips quickly into 50% alcohol to

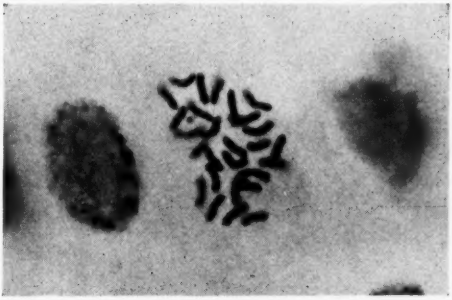

FIG. 2. Mitotic figure of aneuploid orchard grass with 27 chromosomes, × 2000.

harden the glue. From this point they are passed through the alcohol series to paraffin as suggested by Randolph[5] with one addition. The last change of the normal butyl alcohol is colored a medium red with safranin to stain the root tips for orientation during embedding and identification at sectioning. From 20 to 30 cards are placed on a circular flat wire tray (hardware cloth with one-fourth inch mesh) about 9.5 cm. in diameter which fits into covered preparation dishes. The alcohols may then be changed merely by transfer of the tray and cards to dishes containing the desired solutions.

With the colored normal butyl alcohol, the cards are moved to small beakers containing solidified paraffin (56° to 58° C. melting point) to a depth of 6 to 8 mm., and set into the paraffin oven overnight. An early morning change to melted paraffin leaves them ready

[5]Randolph, L. F. A new fixing fluid and a revised schedule for the paraffin method in plant cytology. Stain Techn., 10, 95–6. 1935.

for embedding in 3 hours. Since root tips of the grasses under consideration are of small diameter and frequently crooked, it is necessary in some cases to rearrange them during the embedding process so that they lie nearly parallel and close together. Then they may be sectioned transversely with fair accuracy and uniformity. It may be necessary to break them from the card to secure this orientation. Orchard grass and fescue are microtomed at 12 μ.

The chromosomes in the accompanying figure (Fig. 2) were stained by the Feulgen reaction following the suggestions of Coleman[6] in preparing the stain and of Warmke[7] in its application. The time required for hydrolysis previous to staining varies with the material. For orchard grass, hydrolysis for 23 to 25 minutes is the optimum length of time, while for meadow fescue, 30 minutes results in a better stain. Warmke[7] has suggested the possibility of flattening the chromosomes in a figure by applying pressure to the cover glass, but this is only rarely needed when orchard grass chromosomes have been treated as here indicated. The mounting medium was clarite.

[6]Coleman, L. C. Preparation of leucobasic fuchsin for use in the Feulgen reaction. Stain Techn., 13, 123–4. 1938.

[7]Warmke, H. E. A section-smear method for plant cytology. Stain Techn., 16, 9–12. 1941.

SCHEDULES FOR SECTIONING MAIZE KERNELS IN PARAFFIN[1]

JOHN E. SASS, *Botany and Plant Pathology Section, Iowa Agricultural Experiment Station, Ames, Iowa*

ABSTRACT.—**For paraffin sectioning of maize kernels, the following technic is recommended: Use fresh, turgid kernels and utmost care in removing kernels from the cob and in subdividing into properly oriented slices. Kill and harden in a chrome-acetic-formalin formula. Rinse in water and dehydrate in four grades of dioxane to anhydrous; evacuate with an aspirator and infiltrate with paraffin. If anhydrous dioxane is excessively costly, dehydrate as above to the commercial grade and transfer by intermediate steps to one of the following two-solvent mixtures, using anhydrous ingredients, (A) dioxane and normal butyl alcohol, (B) dioxane and tertiary butyl alcohol, (C) normal butyl alcohol and chloroform, (D) tertiary butyl alcohol and chloroform. Evacuate in the final solvent. (Melted parowax floats on any of the above mixtures, affording gradual, progressive infiltration to pure parowax by periodic decantation and addition of wax.) Finally, transfer to compounded casting wax and cast in paper boats. To prepare a kernel segment for sectioning, fasten to a plastic block, shave the wax from the cutting plane and soak for 12-24 hours, at 35° C., in water containing a trace of safranin or other dye. Mordant starch grains in 1% tannic acid + ½% potassium metabisulphite. A wide choice of simple or multiple stains can be used. These methods are also applicable to tough old stems of corn and hemp, and possibly to many caryopses and seeds.**

The paraffin method can be used to make serial sections of maize kernels as old as 50 days after pollination. The process to be described is based on experience with dent, waxy, flint, sweet and pop corn.

The collecting, sortage, transportation and subdividing of fresh kernels require particular care to avoid dessication or bruising of the kernels. The entire husk-covered ear should be removed from the plant and wrapped in wet paper or cloth, or should stand in a container of water, and be stored in a cool place. The kernels are removed from the ear with a stiff razor blade, one end of which has been broken to form an acute wedge. A row of kernels is cut away on each side of the desired row, and the selected kernels cut away

[1]Journal Paper No. J-1261. Project No. 426.

from the cob, cutting down well into the cob. The removed kernels are kept in water.

The subdivision of the kernel into pieces suitable for processing is a critical operation. The slightest pressure or rolling motion on the kernel results in peeling of the pericarp, disintegration of the endosperm, and dropping out of the embryo during the subsequent processes. Kernels must be selected in which the embryo is straight and oriented along the median line of the kernel. The chaff is removed from a kernel by rubbing gently with the fingers, under water. The kernel is laid on a sheet of wet paper towel, with the germ upward, and a longitudinal slice cut from each side of the kernel. The remaining center slice, which will yield median longitudinal sections, should include little more than the width of the coleoptile and radicle. Then cut off any superfluous cob and pedicel. Drop the piece promptly into the killing fluid. For transverse sections, cut a kernel into transverse slices 2–3 mm. in thickness.

The kernel tolerates considerable latitude in the composition of chrome-acetic-formaldehyde killing fluids. The following is recommended: 1% chromic acid, 30 ml.; 10% acetic acid, 20 ml.; formaldehyde (40%), 10 ml.; 1.0% aerosol (wetting agent), 10 ml.; water, 30 ml.

Each ingredient solution is stable, but the mixture is not stable, and should be mixed immediately before use. However, a stock solution can be prepared, without the formaldehyde, which is added immediately before using. Approximately 50 ml. of the mixture is used for 20 pieces of kernel. The solution is evacuated with an aspirator until vigorous bubbling ceases, then decanted and replaced with fresh solution. Material may be processed after three days in the fluid, but kernels can be stored in the fluid for at least three years. When the tissues are ready for dehydration, they are rinsed with water and covered with the dehydrant fluid.

Dehydration in ethyl alcohol, followed by xylene or chloroform, produces excessive brittleness and is not recommended. Dioxane is a desirable dehydrant because it does not produce such brittleness, permits the use of large changes of concentration, and permits long immersion in any concentration. The following series of aqueous solutions of dioxane has been used most extensively: 10%, 25%, 50%, 75% and anhydrous dioxane, with at least three changes of anhydrous dioxane. With samples of 20 kernels, at least 50 ml. of fluid is used, and an interval of 8–12 hours in each change. The interval in each concentration may be extended to a week, and it is probable that much longer intervals are permissible.

After transferring into the last change of anhydrous dioxane, the material is evacuated with an aspirator. (A quart jar provided with a three-way stop cock serves as a convenient vacuum chamber for exhausting several small bottles simultaneously.) A safety bottle is used containing $CaCl_2$ in the suction line to prevent the entry of water from the aspirator into the specimen jar. The suction is built up until air begins to bubble out of the tissues, then the stop cock on the jar is closed and the evacuated jar allowed to stand until bubbling ceases. The process is repeated until no more bubbles come out of the tissues. This is followed by infiltration in paraffin.

The foregoing schedule seems to be an ideal one, using one reagent as the dehydrant and as the wax solvent. Dioxane may fill this need when a pure, anhydrous product becomes available at an acceptable cost. Distillation of the commercial grade with dehydrating agents has yielded a distillate with a strong aldehyde odor, suggesting the breakdown of some contaminant. A good product can be obtained by more elaborate methods that are still under study.

The advantages of dioxane as a dehydrant can be utilized in conjunction with other solvents. The commercial reagent is satisfactory for the aqueous grades, but a limited quantity of the anhydrous grade must be available. The procedure is as follows: Dehydrate as before in 10%, 25%, 50%, 75% and commercial dioxane, changing the latter twice. Transfer to equal volumes of anhydrous dioxane and normal butyl alcohol, then after 4–8 hours to 1 part dioxane and 9 parts *n*-butyl alcohol. Evacuate and proceed with infiltration.

Tertiary butyl alcohol can also be combined with dioxane in the following schedule: Dehydrate as before in grades of dioxane to the commercial grade, changing the latter twice. Transfer to equal parts anhydrous dioxane and tertiary butyl alcohol. After 4–8 hours, change to 1 part dioxane and 3 parts butyl alcohol. Evacuate and begin infiltration.

A further modification is available if anhydrous dioxane is unobtainable and cannot be prepared conveniently. The addition of 3% chloroform to normal butyl alcohol, or 10% to tertiary butyl alcohol by volume, increases the specific gravity sufficiently to float wax, and accelerates the solvent action. This does not preceptably aggravate the brittleness of tissues. One may proceed as follows: Dehydrate up to commercial dioxane as in the foregoing paragraph. Pass through equal parts of commercial dioxane and either normal or tertiary butyl alcohol, then through three changes of butyl alcohol. Transfer to the chloroform-butyl-alcohol mixture, evacuate and infiltrate.

The progressive method of infiltration used in this laboratory is begun by pouring a teaspoonful of melted household paraffin wax on the surface of the cold solvent in which the specimens are submerged. Equally good results have been obtained with several brands of kitchen wax. If either normal or tertiary butyl alcohol is used as the final solvent, the wax sinks in the solvent and rests upon the tissues. Later in the process, when the wax is brought to the melting point, the melted wax envelopes the partially infiltrated tissues and produces excessively rapid infiltration, a probable cause of distortion of cellular details. Wax floats on the surface of pure dioxane, also on the foregoing two-solvent mixtures, because of the sufficiently high specific gravity of these mixtures. The slow downward diffusion of the dissolving floating wax affords slow infiltration into the tissues.

The solvent is first saturated with wax at 35° C., preferably in a constant temperature oven. If the initial spoonful of wax dissolves completely, another spoonful is added and the material kept at 35° C. until saturation is attained. This is shown by the formation of a translucent crystalline zone on the lower side of the layer of floating solid wax.

The mixture is then transferred to the 52–53° C. oven. The layer of wax melts and remains floating on the solvent. The wax continues to dissolve in the solvent and to diffuse into the tissues. At intervals of 3–4 hours, the bottle is whirled gently to promote homogenization. When the solution no longer shows refraction waves, half the solution is poured off and an equal volume of melted wax added. At intervals of 4–8 hours, it is homogenized, half of the solution decanted, and melted wax added. After three or four such changes, very little solvent is left. All of the fluid is decanted and two or three complete changes of pure kitchen wax are made at 4–8 hour intervals, then a final change is made into a wax-rubber-beeswax compound and the material is cast in paper trays.

Sectioning presents no serious problems. Embedded kernels older than ten days after pollination cannot be cut dry. The cutting of even the youngest kernels is improved by the following treatment: Mount a specimen on a plastic block, and chill. Shave the wax from the cutting plane of the kernel, exposing the tissues. Soak in water at 35° C. for 12–24 hours, with a drop of safranin or other dye in the water to aid in observing the depth of penetration. Cool thoroughly and section with the rotary microtome. Use a heavy knife rather than razor blades for kernels over 10 days old. After sections have been cut for some distance into the material, breakage of the later sections may occur because the warm water had not

penetrated deeply enough. Soak the remaining mounted piece for 12–24 hours and section again.

If the starchy zone crumbles, infiltration has been inadequate, possibly due to incomplete dehydration or insufficient evacuation in the solvent. Incomplete dehydration also results in poor adhesion of wax to external surfaces, and the sections may drop out of the ribbon. If excessive fracturing occurs during the sectioning, and the fault does not lie in the processing, the condition of the knife, the temperature of the room or other traceable factors, the kernels are probably too old and tough to be cut by present paraffin methods.

Sections of old kernels do not adhere well to the slide. Gelatin containing chrome alum and formaldehyde as hardening agents

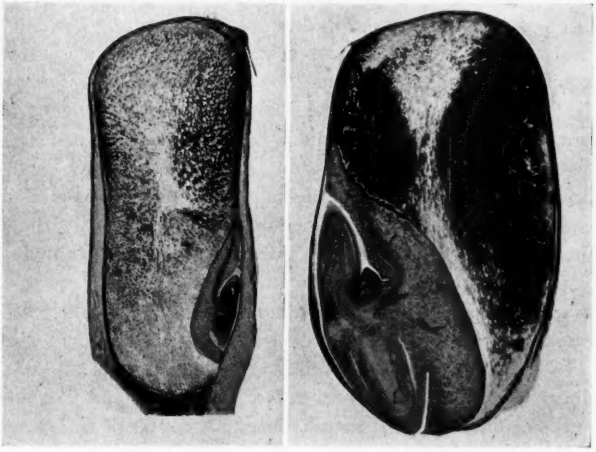

FIG. 1. Kernels of maize, 15 and 42 days after pollination. 8 ×.

has been found to be the most satisfactory adhesive. The ribbon should be fully expanded by warming, and the slides dried for at least 12 hours in the oven at 53° C.

For studies with polarized light, no stain is necessary. However, a light blue stain, obtainable in 2–5 minutes in hemalum, aids in locating the material under the microscope and identifying the region being studied.

The iodine test can be made on paraffin sections, and the characteristic reactions of waxy and ordinary starch are retained. The paraffin is removed with xylene, run down to water through alcohols and stained in aqueous iodine. The slide can be studied or photographed as a temporary water mount or mounted in 20% aqueous

glycerin, which may be allowed to concentrate by evaporation, affording a more durable preparation. When the color has faded out of the starch, the slide should be dropped into a jar of water until the cover slips off, then re-stained with iodine.

The foregoing temporary preparations can be made into permanent stained slides by slipping off the cover glass and proceeding with a staining process. Thus, it is possible to make observations by polarized light, then to use iodine to ascertain the type of starch and its distribution in the kernel, and finally to re-stain the same sections into permanent slides.

Some investigations require the serial sectioning of great numbers of kernels. Many of the slides are discarded or put aside. In such investigations a very simple stain, 15–30 minutes in hemalum, is adequate. Preliminary examination and discarding can be done with water mounts. The desired slides are simply dehydrated and mounted in clarite or balsam. Choice sections that are to be used for more critical study and for projection drawing or photomicrography can be counterstained or re-stained.

The strong chromaticity of tissues of the maize kernel yields brilliant and contrasting staining effects with a wide choice of stains. A satisfactory stain can be obtained with a simple, easily controlled safranin-fast-green conbination. The safranin is retained tenaciously, permitting wide latitude in the timing in the green dye, and any desired intensity of either stain can be obtained. The thin walls of the endosperm cells and the cell walls of young embryos tend to stain weakly. These walls can be accentuated by a light stain in hemalum, obtainable in 1–3 minutes, followed by 2–12 hours in aqueous safranin, and differentiation and counterstaining in fast green. If it is desirable to accentuate the starch grains, they should be mordanted for 30 minutes in 1% tannic acid containing 0.5% potassium metabisulphite ($K_2S_2O_5$) as a preservative, then washed in water and treated with the foregoing stains.

The multiple stains, such as the Flemming triple and the quadruple stains yield beautiful preparations for purposes of display or color photomicrography. However, the relatively simple stains reveal the essential morphological structures adequately for research and afford good differentiation for black-and-white photography.

The foregoing methods are applicable to many other subjects. Tough old corn stems that were formerly cut in celloidin can be ribboned readily. Old stems of hemp, milkweed and dogbane have been cut for fiber studies. It is probable that the caryopses of other grasses, and many difficult seeds and fruits can be sectioned by appropriate adaptations of these methods.

NOTES ON TECHNIC

Preparation of Nematodes for Permanent Microscopic Examination

During the past months, the writer has had some difficulty in preparing horse strongyles (*S. edantatus* and *S. vulgaris*) for microscopic examination. The usual method of clearing specimens in glycerin and mounting in glycerin jelly does not clear thick specimens enough for detailed study. The writer has found the following procedure to give excellent results:

The specimens are removed from the intestine of the host and placed in a beaker of water. The beaker is covered with a layer of cheese cloth and the specimen allowed to wash in running tap water for thirty minutes. This procedure usually removes all debris from the buccal cavity and, also, detaches any debris that may be attached to the bursal rays of male specimens and from the vulva of female specimens. The worms are now killed and fixed in hot 70% alcohol plus 2% glycerin and allowed to remain in this solution for, at least, one hour. The specimens are next placed in 2% H_2O_2 and allowed to remain overnight or until they are bleached white. The specimens are next washed in water for ten minutes and placed in Buxton's media until they are cleared. The length of time for clearing depends upon the thickness of the specimen but, usually, twelve hours will suffice. The specimens are now removed from Buxton's media and mounted in hot glycerin jelly and ringed with clarite, after the jelly has hardened. Specimens mounted in Buxton's media become too transparent after a few months.—J. W. Ward, *Parasitologist, Animal Disease Department, Mississippi Experiment Station, State College, Miss.*

Dried Egg White for Mayer's Albumen Glycerol Fixative

The widely used Mayer's albumen glycerol "fixative" has certain disadvantages, first among which is the apparent necessity for new laid eggs in its manufacture. Recently we tried a reconstituted egg albumen made from commercial dried egg white, and found it very successful. The albumen fixative is made as follows:

Make a 5% solution of dried egg white[1] in 0.5% NaCl solution by shaking at intervals for one day. Avoid frothing. Filter on a small Buchner funnel with vacuum, changing filter paper if filtration stops. One should recover 95% of the solution as clear filtrate.

To 50 ml. of filtered albumen add 50 ml. glycerol and 0.5 ml. 1:10,000 sodium ethyl mercurithiosalicylate. This gives a clear faintly opalescent solution.

Attempts to substitute glycols for the glycerol gave with ethylene glycol a clear solution, with propylene glycol a turbid, milky fluid,

[1]The sample used was obtained from City Chemical Co.

with dipropylene glycol an even milkier opaque white fluid, with diethylene glycol a milky supernatant and a vaguely denser lower stratum, and with triethylene glycol a bulky white precipitate with clear supernatant fluid.—R. R. FAULKNER, *Medical Technician*, and R. D. LILLIE, *Senior Surgeon, U. S. Public Health Service. From the Pathology Laboratory, National Institute of Health, Bethesda, Md.*

TWO NEW BISMARCK BROWNS FOR NUCLEAR STAINING IN FROZEN SECTION TECHNICS FOR FATS

In recent work[1] with oil blue N as a fat stain, the use of Bismarck brown Y and Bismarck brown R (C.I. No. 331 and 332) as nuclear stains was reported.

These basic azo dyes, in common with Janus green B (C.I. No. 133) possess the valuable property of not bleeding into aqueous mounting media such as Apathy's gum syrup. However, the two browns are rather pale in color, particularly Bismarck brown Y. Hence, it seemed desirable to find a deeper color of similar properties. Other dyes, such as the safranins, fuchsins, rhodamine B, and neutral red, have previously been reported as unsatisfactory.

Recently three new dyes[2] related to Bismarck brown were tried. All behaved similarly to Bismarck brown Y and R in their lack of bleeding into the syrup. One, designated as Bismarck brown 53A, gave slightly reddish brown staining of nuclei from a 1% solution in 1% acetic acid. Cytoplasm was only very lightly stained with this or with Bismarck brown 53B. This latter gave slightly yellowish brown nuclear staining. With both of these, nuclear staining was more intense, and cytoplasmic staining less than with Bismarck brown R or Y. The third dye, Bismarck brown 53C, gave a rather disagreeable grayish brown color to nuclei, and was not further tried.

Bismarck browns 53A and 53B are made by coupling two moles of metaphenylenediamine with diazotized 2:4 diaminoanisole by somewhat different processes. They gave absorption maxima at 450 and 452 mμ, respectively. The absorption bands were broad, 90% of maximum density covering a width of 66.3 and 74.5 mμ respectively, with medians at 458.2 and 454.5 mμ. Densities at λ for a 10 mm. cell at 10 mg. per liter dilutions were 0.250 and 0.340 respectively on the Beckman spectrophotometer.

I wish to acknowledge the technical assistance of Medical Technician R. R. Faulkner.—R. D. LILLIE, *Senior Surgeon, U. S. Public Health Service. From the Pathology Laboratory, National Institute of Health, Bethesda, Md.*

[1]Lillie, R. D. Oil blue N or NA as a fat stain in animal histology. Stain Techn., 20, 7–9, 1945.

[2]The samples of Bismarck brown 53A, 53B, and 53C, and data as to their structure, were furnished by the National Aniline Division.

LABORATORY HINTS
FROM THE LITERATURE

A DEPARTMENT DEVOTED TO ABSTRACTS OF BOOKS AND PAPERS FROM OTHER JOURNALS DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

MICROSCOPE AND OTHER APPARATUS

CLARK, GEORGE L., BAYLOR, MARTHA BARNES, MARTIN, DOROTHY E., and RAFFERTY, GERTRUDE T. **The electron microscope in dermatology.** *Arch. Dermat. & Syph.*, **51**, 81–9. 1945.

Electron micrographs of the skin and other types of tissues, of cell constituents, of single protein molecules and viruses, and of cosmetics are reproduced and discussed. Fixing and staining technics are described.—*J. A. Kennedy.*

DYES AND THEIR BIOLOGICAL USES

DOMM, L. V., and BLIVAISS, B. B. **Modification in feather pattern and growth following administration of thiouracil in Brown Leghorn fowl.** *Proc. Soc. Exp. Biol. & Med.*, **57**, 367–8. 1944.

Feeding Brown Leghorn capons 0.3–0.5 g of thiouracil daily induced a change from black to red pigments in feathers. Thyroidectomy has the same effect. Feeding thiouracil produced hypertrophy of the thyroid accompanied by the signs and symptoms of thyroidectomy.—*M. S. Marshall.*

HETHERINGTON, DUNCAN C. **Effect of trypan blue upon cardiac explants in tissue culture.** *Proc. Soc. Exp. Biol. & Med.*, **57**, 194–5. 1944.

Heart tissue from chicken embryos transplanted to media containing trypan blue showed significantly faster growth rates than explants in control medium. Accelerated uptake of oxygen is the hypothetical explanation.—*M. S. Marshall.*

ANIMAL MICROTECHNIC

BAKER, JOHN R. **The structure and chemical composition of the Golgi element.** *Quart. J. Micr. Sci.*, **85**, 1–71. 1944.

The following fixative is recommended: formalin (formaldehyde, approx. 40%), 10 ml.; $CaCl_2$, anhydrous, 10% aqueous, 10 ml.; distilled water, 80 ml. Some precipitated chalk is kept in the bottle with the solution. If soaking for more than 3 days is desired, the fixed material is transferred to a solution similar to that just described except that 10 ml. of water is replaced by an equal amount of 10% aqueous $CdCl_2$ solution.

After washing in running water, the tissue is embedded in gelatine and sectioned on the freezing microtome. Staining is by means of a saturated solution of Sudan black in 70% alcohol; counterstaining is by Mayer's carmalum. Final mounting is in glycerin jelly or levulose syrup.—*C. E. Allen.*

DOETSCHMAN, WILLIS H. **Some suggestions in microtechnique particularly useful in microentomology and parasitology.** *Trans. Amer. Micr. Soc.*, **63**, 175–8. 1944.

The author discusses a method to kill, fix, dehydrate, clear, stain, and mount a specimen all in one operation, using the following modification of Berlese's medium: water, 35 ml.; chloral hydrate, 20 g.; gum arabic, 20 g.; glycerin, 20 ml.; glucose syrup, 3 ml.; basic fuchsin, 10 drops or more (0.3 g. per 10 ml. 95% alcohol). The formulae for the Berlese type of media allow for considerable variation. Poor results with one group of anthropods may warrant some changes in the ingredients. Highly chitinized specimens should be treated with 10% KOH from overnight to 24 hr. Oven treatment at 37°C. to 50°C. from a few hours to one day will give a permanent mount. With this medium crystallization is absent and prepared slides are resistant to sunlight.

Air bubbles may be avoided by placing specimen in a small drop of medium on a slide, putting a drop of medium on the coverslip and inverting. Gentle pressure will cause bubbles to flow out. The use of glass bridges is recommended to protect the specimen.—*Challiss Randall.*

DUNN, R. C., and THOMPSON, E. C. **A new hemoglobin stain for histologic use: a slightly modified Van Gieson stain.** *Arch. Path.*, **39**, 49. 1945.

The staining procedure is as follows: Bring sections to water in a glass staining dish; stain 15 min. in 0.25% hematoxylin in 5% aqueous alum; wash in tap water, and immerse for 1 minute in 4% aqueous ferric ammonium sulfate; wash lightly in tap water; stain 10 minutes in above aqueous alum-hematoxylin; rinse quickly in tap water; stain 15 minutes in Van Gieson's solution (13 ml. of 1% acid fuchsin, 87 ml. of saturated aqueous picric acid); transfer to 95% ethanol for 3 minutes; clear in xylene and mount in clarite.

For routine use, neutral buffered formaldehyde solution is recommended as fixative, but for bone marrow and spleen Zenker's solution or Helly's fluid is better. Prolonged standing in formaldehyde solutions impairs hematoxylin staining of hemoglobin.

In red blood cells, kidney casts, reticuloendothelial cells of spleen, liver and bone marrow and epithelial cells of convoluted tubules of kidney, hemoglobin stains a green color, varying from light olive green to deep pea green or blackish green, depending on the hemoglobin content. Slides stained in this manner keep for at least 6 months in the dark without fading. Collagen stains red by this technic.—*L. Farber.*

HIGHMAN, B., and LILLIE, R. D. **Romanowsky staining with buffered solutions. IV. Influence of fixatives on buffer requirements.** *J. Tech. Methods and Bull. Internat. Assoc. Med. Museums*, **24**, 31–6. 1944.

Quite satisfactory differential tissue staining can be attained with most fixatives by selecting appropriate pH levels. The staining solution used by the author was prepared as follows: stock eosinate of polychrome methylene blue, 20 ml. (0.6 g. Wright's stain, N.I.H. Lot HO, in 100 ml. each of glycerol and methanol or 0.2% eosin B, azure A in glycerol methanol); C.P. methanol, 30 ml.; C.P. acetone, 30 ml.; distilled water, 300 ml. This stain was distributed in 10 Coplin jars to each of which was added 2 cc. mixed buffer solution of graded pH levels. Two series of buffer solutions were used. The first series consisted of M/15 citric acid and M/15 anhydrous Na_2HPO_4 each in 25% methanol and the second of 25% methanol solutions of M/10 citric acid and M/5 Na_2HPO_4. Satisfactory nuclear staining of liver and kidney was required as the limit on the acid side, pink staining of smooth muscle on the alkaline.—*C. Randall.*

KOONZ, C. H., and STRANDINE, E. J. A rapid and simplified method for revealing the surface pattern of hair. *Trans. Amer. Micr. Soc.*, **64**, 63–4. 1945.

The scale pattern, especially of fine hairs, may be demonstrated by the following method: smear a clean cover glass with a thin layer of glycerin, place the hair on the cover glass, cover it with a drop or two of water soluble nigrosin solution, tilt the cover glass to remove the excess fluid by tipping it with blotting paper, dry the preparation over gentle heat, remove the hair, invert the cover glass and place it on a slide for microscopic examination.— *Virgene Kavanagh.*

LEACH, E. H. **Post-fixation of tissues.** *J. Path. & Bact.*, **57**, 149–52. 1945.

After tissue has been fixed by any desired method and dehydrated and cleared with xylene, improved fixation with consequent better staining is secured by treating the tissue with absolute alcohol (one change) 6–24 hr. This is the "post-fixation" stage. Apparently some of the protein is protected from fixation until the fat is removed. Granules stain sharper. Cell boundaries, intracellular membranes, mucoproteins and mitochondria are better preserved and stain more sharply. Phosphatase by Gomori's method and vitamin C by Barnett and Bourne's method show improved fixation.—*S. H. Hutner.*

SAMUEL, D. M. **The use of an agar gel in the sectioning of mammalian eggs.** *J. Anat.*, **78**, 173–5. 1944.

Stock agar which should be filtered and which may be preserved indefinitely by the addition of 5% formaldehyde is made in two strengths, 0.65% and 1.3%. A thin film of 1.3% agar is allowed to set firmly on a slide and the egg added to it, either directly from fixative or after being taken down to water. A drop of the 0.65% agar is now placed near the egg and allowed to envelop it and to set firmly. A further thin film of 1.3% agar is now added. Orientation of the egg can be effected before the 0.65% agar has set, by means of a hair held in artery forceps. Dehydration of the agar "sandwich" with its contained egg should be carried out gradually. After absolute alcohol the piece is taken into a mixture of equal parts of methyl benzoate and a saturated alcoholic solution of eosin. An oil immersion lens can be used now to examine the egg. The optimum direction for sectioning can be indicated by cutting a facet on the agar piece. This agar piece, the new shape of which should be recorded, is then carried through benzene, benzene wax, and paraffin-wax before being "blocked out". Since the outline of the agar is seen readily in the cold wax, proper orientation to the microtome knife can be made. Such a method should be useful for other small objects as well as eggs.—*Warren Andrew.*

STOWELL, R. E., and STOKES, J. M. **Glyoxal and furfural as substitutes for formaldehyde in tissue fixation.** *J. Tech. Methods and Bull. Internat. Assoc. Med. Museums.*, **24**, 25–30. 1944.

The authors report that furfural is not a satisfactory substitute for formaldehyde as a general fixative for tissues. Neutralized methyl glyoxal preserves red blood cells, whereas a stock solution which is acidic would destroy them, but it does not give as satisfactory fixation of tissues as formaldehyde. Glyoxal gives less differential shrinkage of tissues and better cytoplasmic preservation of tissues than formaldehyde if used in concentrations of 2 to 6%. However, since glyoxal is less satisfactory than formaldehyde as a preservative for gross sections and is not a good substitute in Zenker's or Bouin's fluids, it can be recommended as a general substitute for formaldehyde in tissue fixation only where there is a formaldehyde shortage.—*C. Randall.*

MICROÖRGANISMS

MULLER, HUGH E., and CHERMOCK, RALPH L. **A rapid staining technique for acid fast organisms.** *J. Lab. and Clin. Med.*, **30**, 169–71. 1945.

The recommended procedure is as follows:
To stain smears, fix the preparations with heat and stain 1 min. with modified Kinyoun's carbol fuchsin (basic fuchsin 4 g., phenol crystals 8 g., 95% alcohol 20 ml. and distilled water 100 ml. Just prior to use add 1 drop of Tergitol No. 7 to each 30–40 ml.). Decolorize 30 sec. (HCl 3.0 ml. and 95% alcohol 97 ml.). Counterstain 1 min. in a solution of 1% aquous KOH 0.07 ml., distilled water 70 ml., methylene blue 30 ml. (1.48 g. in 100 ml. of 95% alcohol). Rinse with water and dry. To stain tissue sections, fix the tissue 24 hr. in 10% formalin, embed in paraffin and cut. Place sections in xylene 3 min., second xylene 2 min., 95% alcohol 2 min., 80% alcohol 2 min., 70% alcohol 2 min., 50% alcohol 2 min., 30% alcohol 2 min., and distilled water 2 min. Place in the above fuchsin stain 5 min., rinse with distilled water, decolorize with alcohol till the section is a light pink, counterstain in fast green (FCF?) 2 min. (1.5 ml. saturated alcoholic fast green and 98.5 ml. of distilled water), rinse with water, place in 95% alcohol 1 min., absolute alcohol 1 min., xylene 2 min., second xylene 5 min. and mount in balsam or dammar. The method is rapid and equal to, or better than the older procedures.—*John T. Myers.*

WISSEMAN, CHARLES L., JR. **Rapid staining method for relapsing fever spirochetes.** *Science*, **101**, 392. 1945.

A method is described for applying the modification of the Field stain of Medalia *et al.* to the staining of the spirochetes of relapsing fever. Thick films are prepared and dried in the usual manner and laked in distilled water or 2% acetic acid, after which they are stained in essentially the same manner as de-

scribed by Medalia *et al.* The spirochetes stain a deep purple. Actual staining time is 10 to 15 sec. The method has been used successfully in both animal and human infections of relapsing fever.—*T. M. McMillion.*

WORLEY, GORDON, JR., and YOUNG, GERALD. **The glanders organism with reference to its cell inclusions.** *J. Bact.*, **49**, 97–100. 1945.

The irregular staining of *Malleomyces mallei*, referred to frequently in the literature, is considered to be due in a large part, if not entirely, to the presence within the protoplasm of lipoidal granules which fail to stain readily with the usual aniline dyes. Such granules do, however, stain readily with Sudan black B or with the iodine-fuchsin procedure for staining lipid bodies. They are deposited in cells whether grown on plain agar, tryptose agar, or glycerol glucose agar. Attempts to demonstrate the presence of volutin were unsuccessful.—*Gordon Worley, Jr.* (Courtesy *Biological Abstracts*).